Sight-threatening Uveitis:
a 2-way Street between Research and Clinic

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Autoimmune uveitis:
Lessons from animal models

Animal models can give us invaluable insights into disease processes but they cannot mimic human disease perfectly.

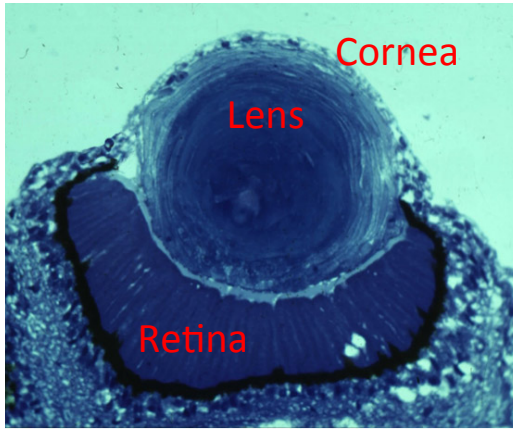
We need to understand the similarities and the differences, so as to apply these insights intelligently to designing rational therapies.

The eye: an 'outpocketing' of the brain, or vice versa?

- In evolution, an organized eye precedes an organized brain

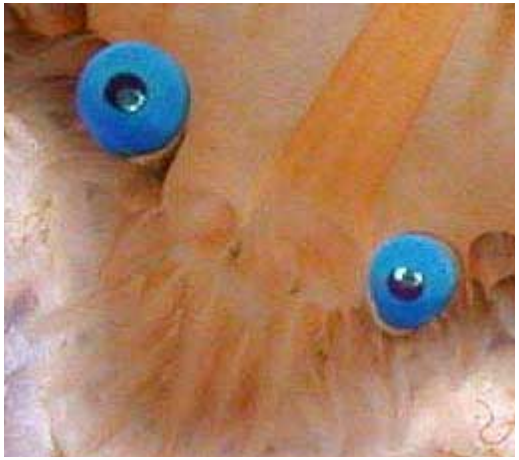
Jellyfish

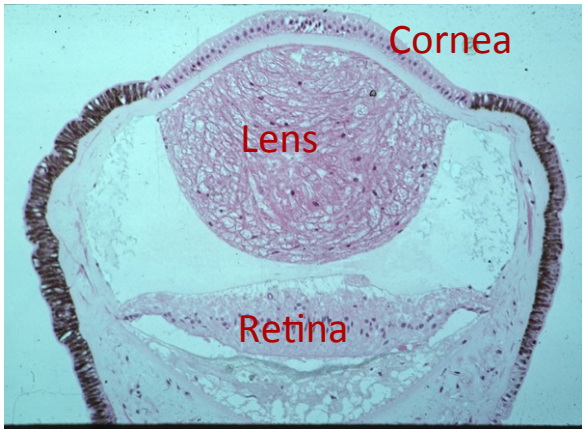
Monterey Aquarium 2013



Piatigorsky et al. J. Comp. Physiol. 1989

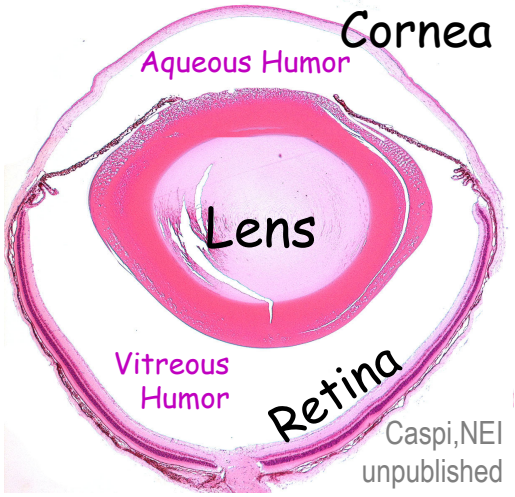
Scallop





Piatigorsky et al. J. Biol. Chem. 2000

Mouse



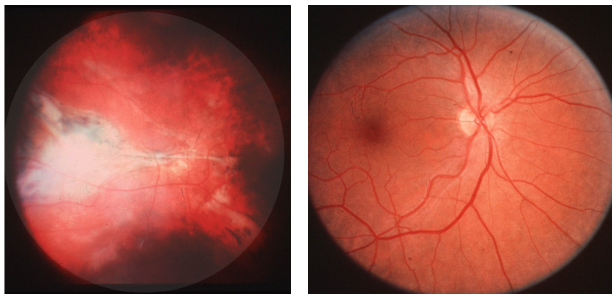
Caspi,NEI unpublished

Ocular immune privilege

- **Vision is a very strong evolutionary selective pressure: any reduction in visual function affects survival**
- **The eye has evolved strategies to limit inflammation resulting from day-to-day stresses and insults to preserve vision**
- **Clinical: corneal transplantation – 90% acceptance by 1 yr**

Separation	**Blood-Retinal Barrier:** prevents free traffic of cells and even large molecules into and out of the eye. No lymphatic drainage (as long as BRB is intact)
Inhibition	**Immuno-inhibitory ocular microenvironment:** soluble factors in ocular fluids - TGFβ, αMSH, VIP, CGRP; RA cell-bound molecules - FasL, TSP1, PD-L1,etc Few (and quiescent) potential APC in the healthy retina
Regulation	**Eye-driven systemic regulatory processes** (ACAID and post-recovery eye-dependent tolerance)

Human autoimmune uveitis "non-infectious uveitis"



USA	Incidence	52.4 / 100,000
	Prevalence	115.3 / 100,000

Accounts for 10–15% of blindness

Restricted to the eye

- *Idiopathic uveitis*
- *Sympathetic Ophthalmia*
- *Birdshot chorioretinipathy*

Part of systemic syndrome

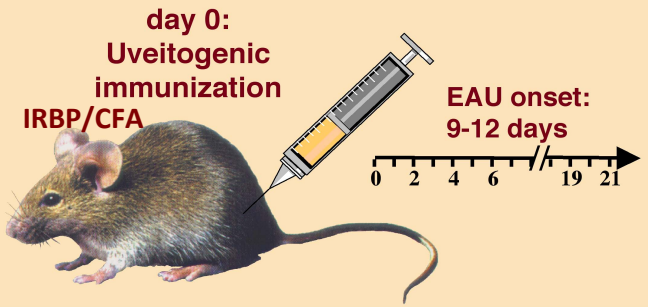
- *Sarcoidosis*
- *Behcet's disease*
- *Vogt-Koyanagi-Harada disease*

- ✓ Sterile inflammation within the eye
- ✓ Strong MHC associations, immune responses to retinal proteins (arrestin, IRBP)
- ✓ Disease-associated Th1 and Th17 responses, which may explain heterogeneity
- ✓ Improvement with T cell targeting agents (CsA, rapamycin, anti-IL-2R)

- ➢ **Retina-specific T lymphocytes may be involved in driving disease pathology**
- ➢ **Autoimmune or Autoinflammatory?** (genetic association with inflammasome genes, e.g., NLRP3 and Behcet's disease, & involvement of IL-1 in pathogenesis)
- ➢ **Irrespective of the initial etiology, autoimmune responses to retinal Ags released as a result of tissue breakdown may become drivers of disease** (e.g., responses to retinal arrestin reported in "autoinflammatory" Behcet's uveitis).

Experimental autoimmune uveitis (EAU): a model for human uveitis

Caspi et al, J. Immunol 1988



day 21: Assess EAU & immunological responses

Induced by immunization with retinal proteins recognized by lymphocytes of uveitis patients

IRBP *(interphotoreceptor retinoid binding protein)*
Some strains: **retinal arrestin** *(retinal soluble Ag = S-Ag)*

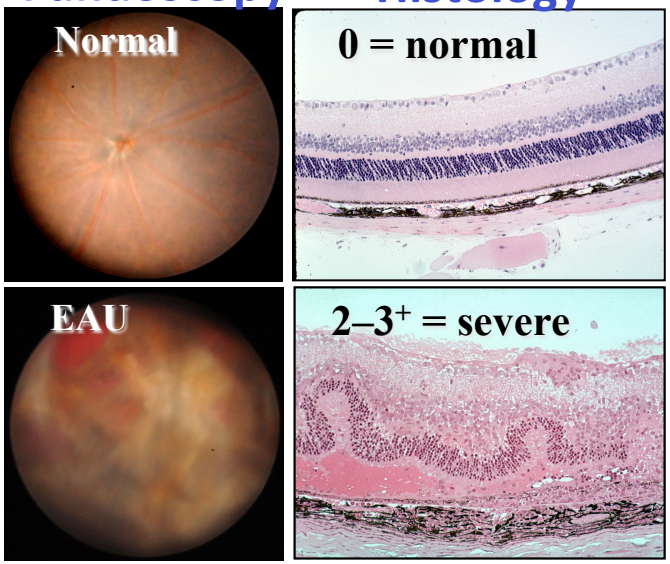
Both Th1 and Th17 cells are induced

Quantitation of disease: Scored on a scale of **0 – 4** by type and number of lesions

Susceptibility is strain-dependent

B10.RIII > C57BL/6 > BALB/c

Fundoscopy **Histology**

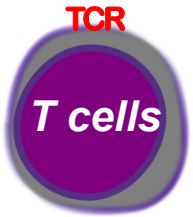


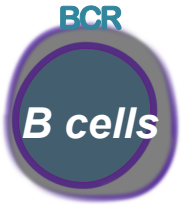
"Classical" model of tissue-specific autoimmunity: shares basic mechanisms with EAE, CIA, AIO, etc

Some basic stuff about immunity and autoimmunity

- Our immune system has evolved to deal with pathogens.
- There are millions of different microbial components (antigens) to which immunity can be generated.
- Our own bodies produce >100,000 different proteins: how can the immune system produce a broad repertoire against pathogens, while at the same time avoiding reacting to self?
- Lymphocytes are **selected** during an immature stage of development so that the ones which can respond to self are eliminated.
- When this process fails, the result is **autoimmunity**: an inappropriate response of the immune system to self components.
- Lymphocytes (T and B cells) and antibodies specific to self components find their target antigens and cause inflammation and damage in the tissue.

In uveitis, T cells appear to have a central role





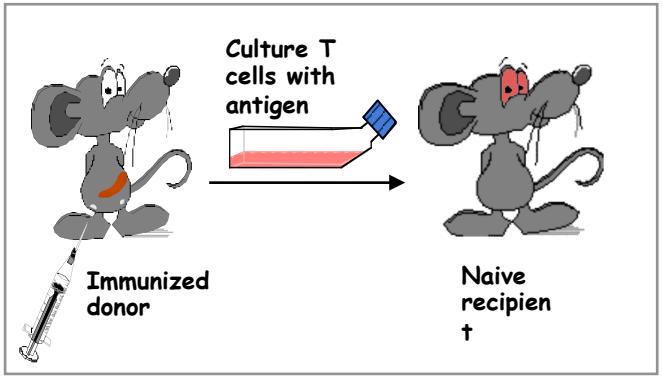
Biological role

Recognition of antigen (via TCR)
Killing of cells by direct contact
Production of soluble and cell-bound mediators that call in inflammatory leukocytes and help B cells make antibodies

Recognition of antigen (via BCR)
Production of antibodies that bind to antigens on cells surface and activate leukocytes and complement

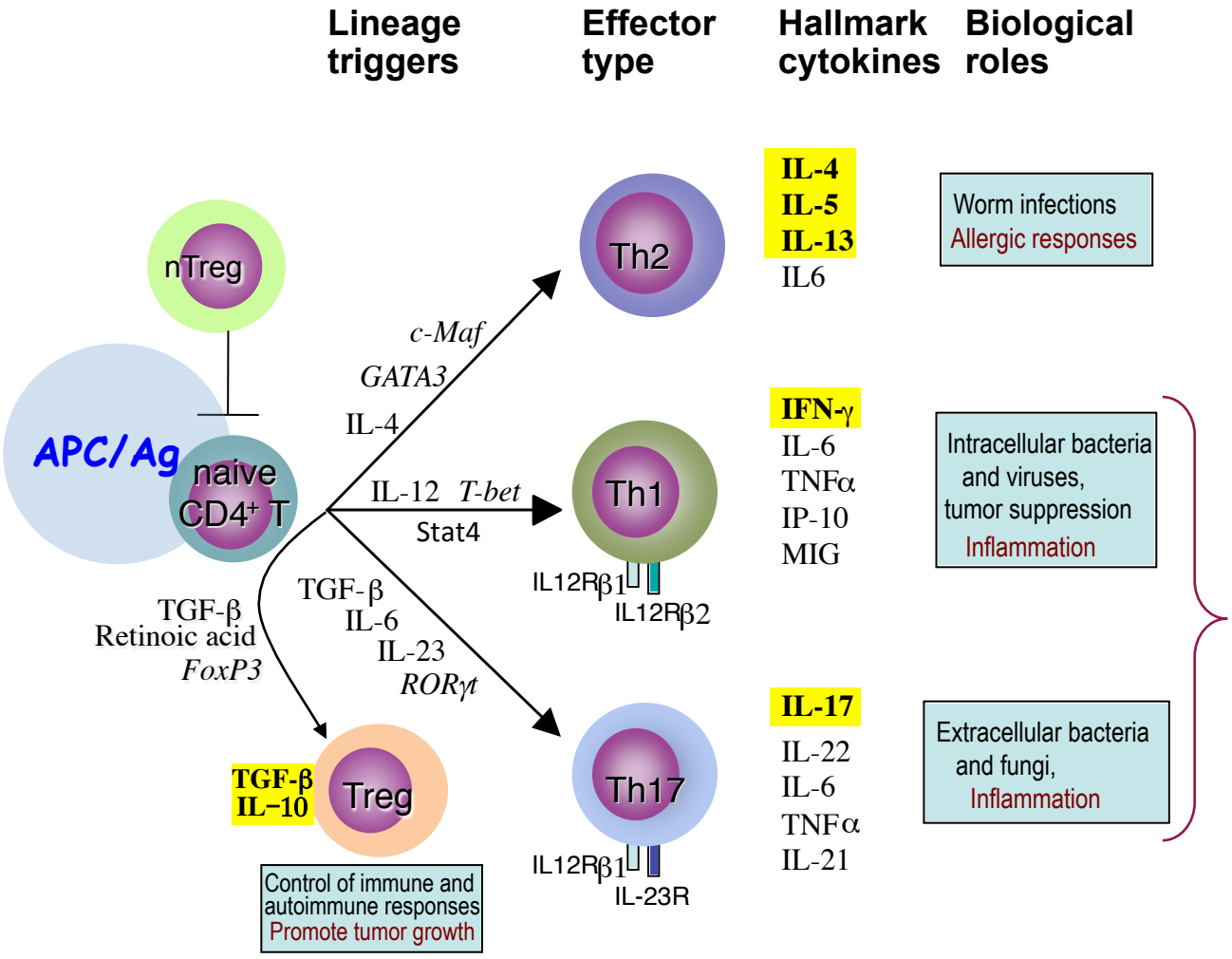
EAU is a T cell-dependent disease

- EAU cannot be induced in T cell-deficient animals
- is suppressed by T cell-targeting agents (e.g., CsA, rapamycin, anti- IL-2R Ab)
- transferred from immunized donors to normal recipients by T lymphocytes, not by serum



- To understand EAU, we must study the T cells involved

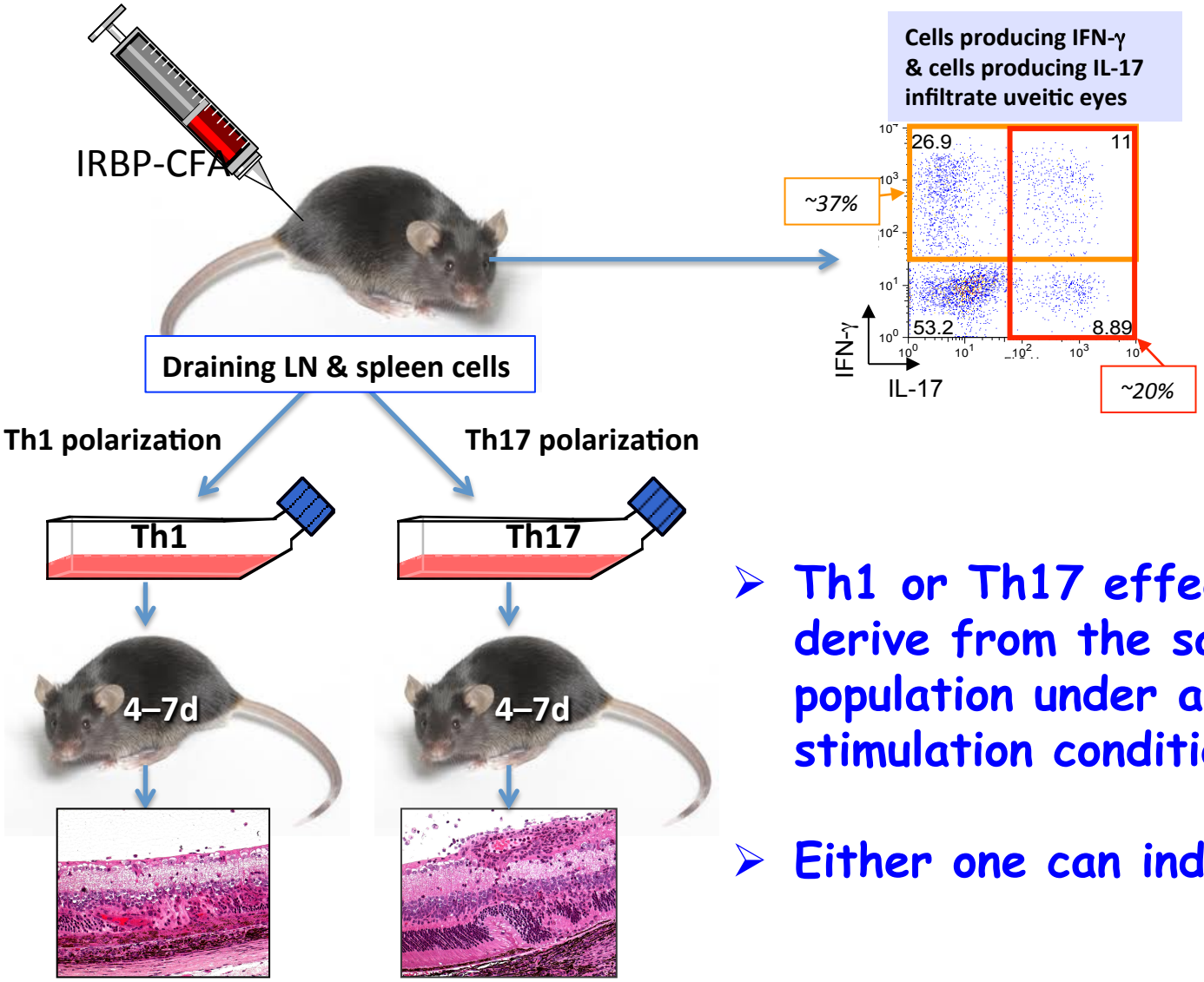
Antigen-specific T cells:
distinct effector types to deal with distinct pathogens



Naïve T cells exposed to their Ag for the first time differentiate into one of 3 major effector lineages: Th1, Th2 or Th17, depending on the nature of the environmental cues present during their activation (provided by the pathogen)

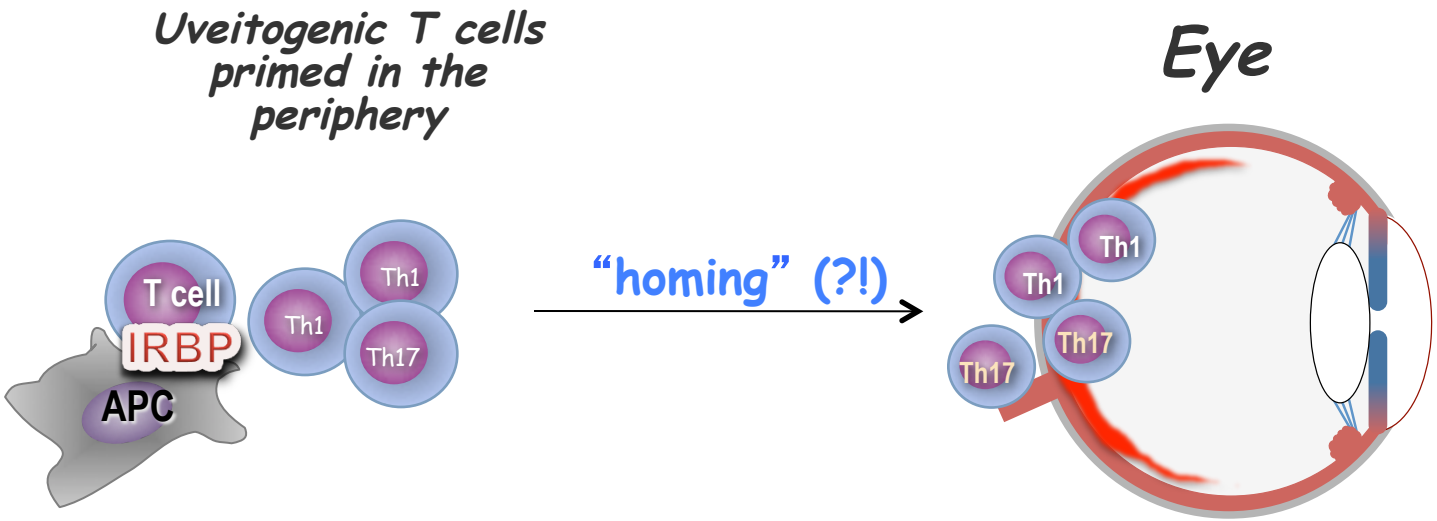
Inflammatory autoimmune diseases such as uveitis, multiple sclerosis, type 1 diabetes, and rheumatoid arthritis

What kind of T cells induce uveitis?



- Th1 or Th17 effector T cells derive from the same population under appropriate stimulation conditions
- Either one can induce uveitis

How do we get there from here?



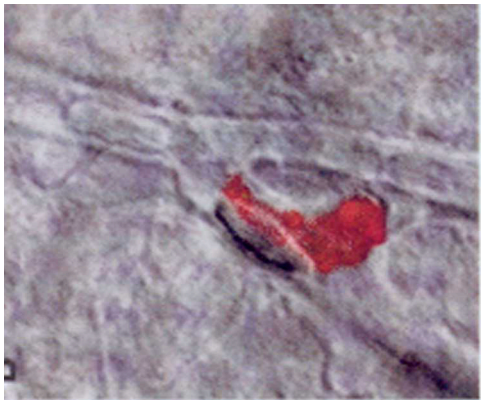
Entry of the first activated T cells into the intact eye is accidental

In situ recognition of antigen is a prerequisite for disease induction

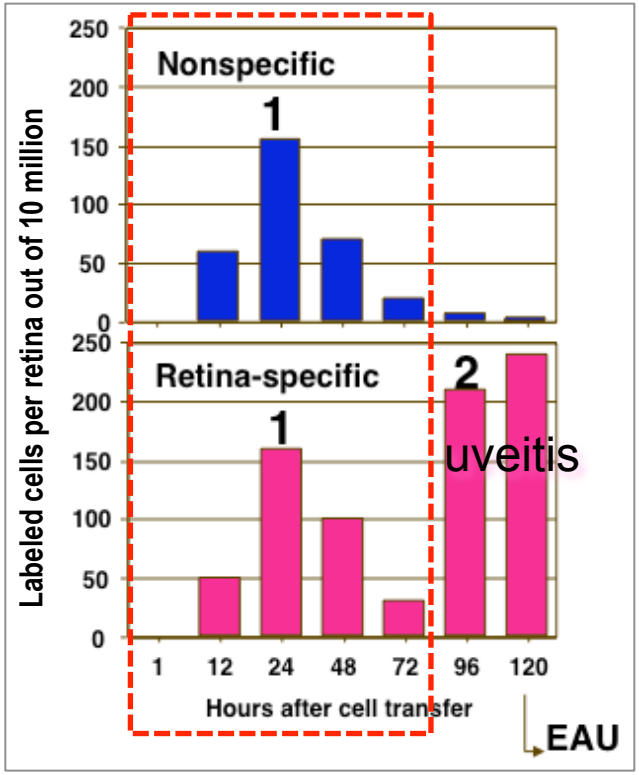
Recruitment of inflammatory leukocytes required for disease progression

- Unlike naïve T cells, which are excluded from the eye, activated T cells can actively penetrate the BRB. They are 'tissue-seeking': they express adhesion molecules, produce matrix-degrading enzymes, can stick to blood vessels and extravasate into tissues. **Blood-tissue barriers cannot stop them.**

T cell infiltrating the retina



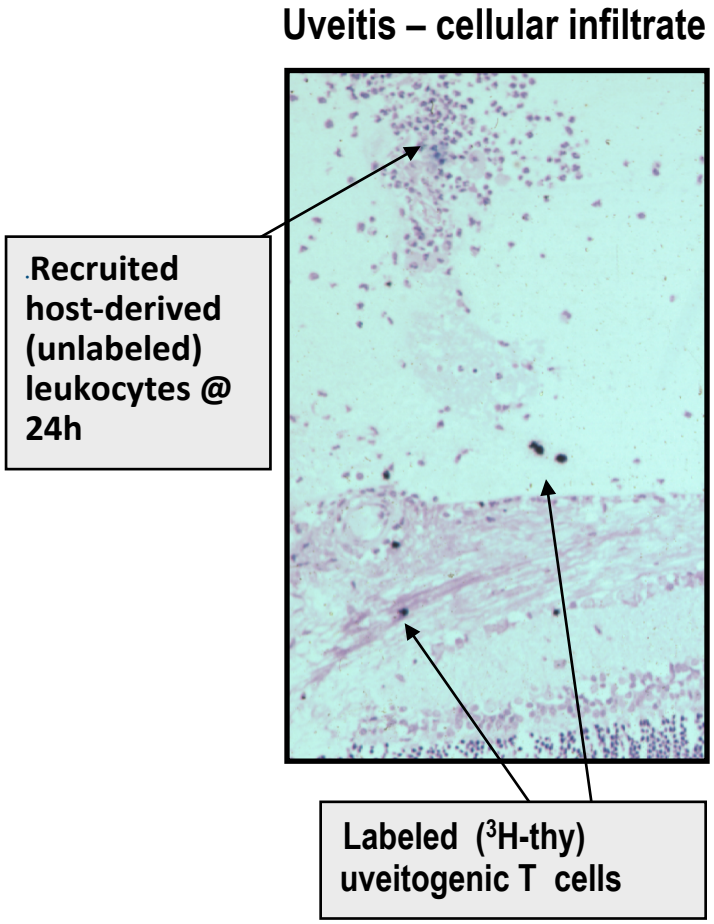
- **Circulating activated T cells that reach the eye, do so at random.**
- **Experiment**: inject 10^6 labeled, activated retina-specific or nonspecific T cells into rats and count all cells that enter the retina.
- **Result:** specific and nonspecific T cells enter the retina equally, but only the specific ones induce uveitis. => Induction of disease requires antigen recognition within the eye



(Prendergast et al. IOVS 1998)

Retina specific T cells don't do it alone:
critical role of recruited leukocytes in pathogenesis

- **Injection of small numbers of activated retina-specific T cells into the eye triggers massive recruitment of inflammatory leukocytes and induction of EAU**
- **Physical damage to the tissue is mediated by leukocytes recruited from the blood**



Kim et al, Cell. Immunol. 1988

How many Ag-specific lymphocytes are needed to induce EAU?

- **150 out of 10 million labeled eye-specific lymphocytes enter the retina**
- **Fewer than 0.5 million cells are needed to induce EAU**

$$\frac{150}{x} \quad \begin{matrix} — \\ — \end{matrix} \quad \frac{10{,}000{,}000}{500{,}000}$$

$$x = 7.5$$

Healthy people have circulating T cells specific to ocular antigens that can cause uveitis

Frequency of T cells specific to retinal arrestin (S-Ag) in PBL:

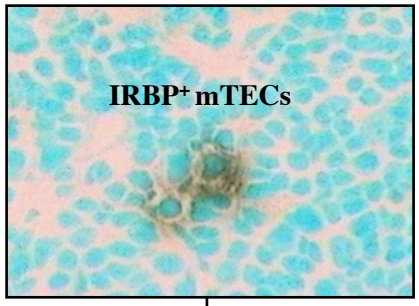
- 4 / 10^7 lymphocytes by proliferation (de Smet et al, OII 1998)
- at least 20x underestimation *vs.* methods using MHC-tetramers (Tan et al, JI 1999).
- at least 10 antigens known to be uveitogenic in animals.

≥ 80 eye-specific T cells per million lymphocytes

S-Ag (retinal arrestin)
IRBP
Rhodopsin
Opsin
Phosducin
Recoverin
Rpe65 (RPE)
Melanin (iris, choroid)
TRP1 & TRP2 (choroid)
Lens proteins

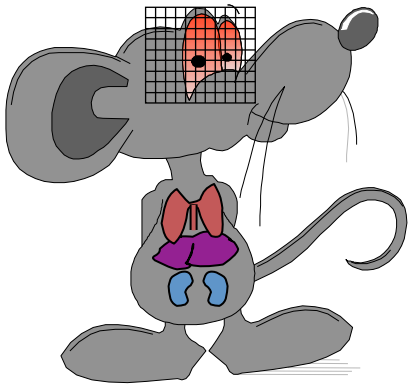
Why do we have so many T cells that recognize retinal Ags?

Central tolerance
Repertoire selection by retinal Ag expressed in the thymus



"Escapees" → Thp

~~Peripheral tolerance~~



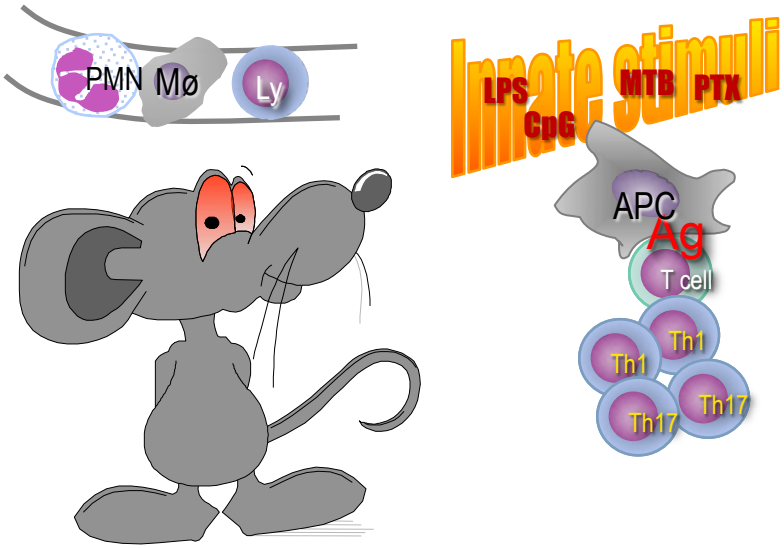
BUT…

SO…

- **Tissue Ags are expressed in the thymus under control of the AIRE protein**
  –> *this includes retinal Ags* *(Egwuagu and Gery – 1997, 1998)*
- **Most retina-specific T cells are deleted in the thymus**
- **The thymic selection process is not 100% efficient. "Escapees" would normally be tolerized in the periphery by contact with self Ags**
- **Sequestration of retinal Ags behind the blood-retinal barrier (BRB), in the absence of their expression outside the eye, hinders peripheral tolerance**
- **Retina-specific T cells persist in the circulation in an "ignorant" but not tolerant state, and can be activated by a chance encounter with a retinal Ag (trauma to the eye), or an unknown (microbial?) mimic.**
- **Following activation, they acquire the ability to actively break through the blood retinal barrier. They recognize their Ag in the tissue, recruit inflammatory leukocytes from the circulation, and elicit uveitis.**

Induction and development of uveitis

Circulating retina-specific T cells are activated by a chance encounter with a retinal Ag (following trauma to the eye), or an unknown microbial mimic. They:



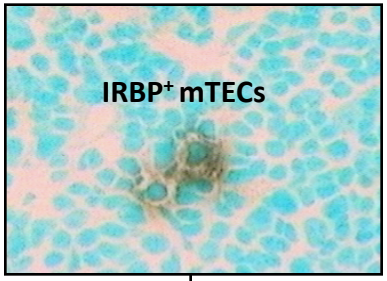
- proliferate and differentiate into activated effector cells
- travel to the eye and actively cross the blood-retinal barrier
- recognize their cognate Ag in the tissue, whereupon they secrete proinflammatory cytokines that activate the vascular endothelium and recruit inflammatory leuko-cytes from the circulation, resulting in uveitis

Critical checkpoints in pathogenesis of uveitis

Why?

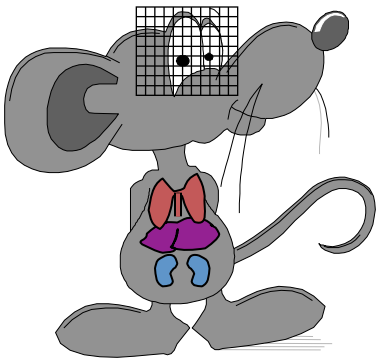
Central tolerance

Repertoire selection by retinal Ag expressed in the thymus



"Escapees" Thp

Peripheral tolerance



Why not?

"natural" T-reg cells

nT-reg

spleen

"Induced" T-reg cells

T-reg

F4/80+ APC, ???

Presentation of antigen to T cells in the periphery

T cell

Ag

APC

Innate stimuli

LPS CpG

MTB PTX

Activation and clonal expansion

Proinfl. Cytok. IL-12, IL-23 IL-6, TGF-β

Th1

Th1

Th17

Activated uveitogenic T cells

Migration and infiltration into the eye

Chemokines, Adhesion mol.

UVEITIS

Recruitment of leukocytes from circulation

Chemokines, Adhesion mol.

PMN Mø Ly

How?

Autoimmunity in the eye: the "price of privilege"?

Peripheral tolerance is impeded due to relative sequestration of retinal Ags as part of immune privilege.

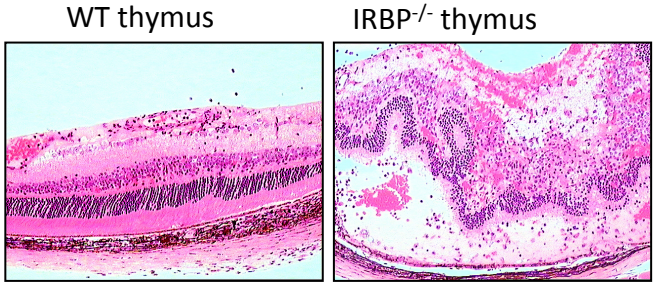
Consequently, the eye seems overwhelmingly dependent on the thymus as the main mechanism of self tolerance

Dody Avichezer

Mice that fail to eliminate retina-specific T cells in the thymus are more susceptible to uveitis

(Avichezer et al., J Exp Med 2003)

EAU in transplant recipients of:

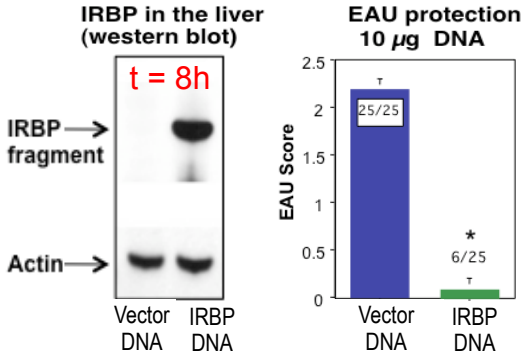


Phyllis Silver

Conversely, forced expression of retinal antigens in peripheral tissues, in a non-privileged environment, induces immune tolerance and protects from uveitis

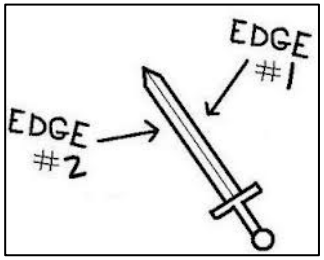
(Silver et al., J Immunol 2007)

Hydrodynamic injection of IRBP plasmid



Does ocular immune privilege do anything to protect us from uveitis?

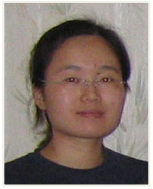
Sequestration behind Blood-Retinal Barrier is a double-edged sword: although it largely excludes circulating leukocytes from the healthy eye, it hinders establishment of peripheral tolerance



The ocular microenvironment is immuno-inhibitory:
- soluble factors in ocular fluids - TGFβ, αMSH, VIP, CGRP; RA
- cell-bound molecules - FasL, TSP1, PD-L1,etc
- Culture of lymphocytes with ocular fluids or with some types of ocular cells can **convert T cells to Foxp3+ Tregs**

Evidence from *in vitro* studies

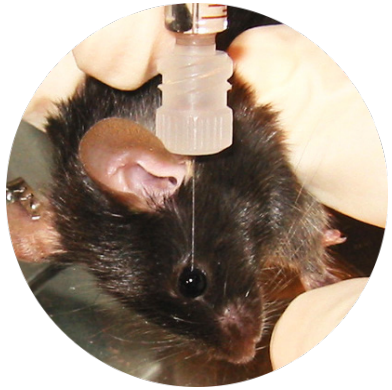
What happens *in vivo* to retina-specific T cells that enter the eye?


Ru Zhou

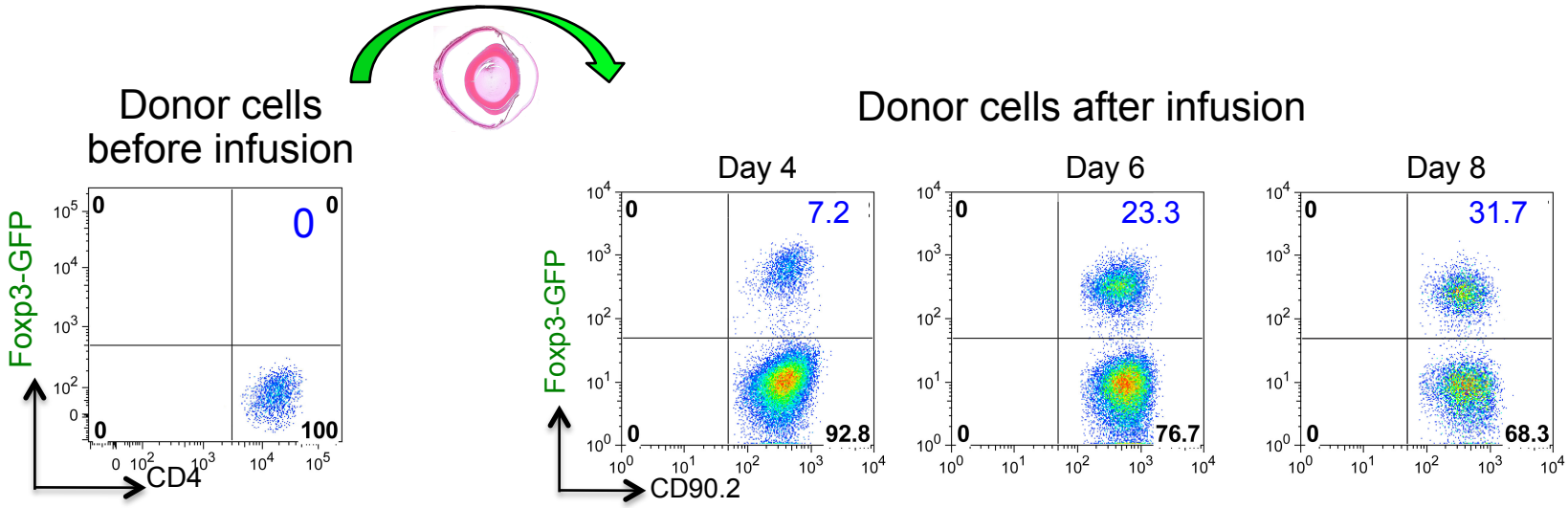

Reiko Horai

Inject sorted Foxp3$^-$ retina-specific T cells* into the living eye and examine induction of Foxp3

* IRBP TCR Tg, Foxp3-GFP reporter, CD90.2$^+$
—> Can follow donor cells in recipient eyes whether or not they convert to Tregs



Naïve retina-specific T cells injected into the living eye are converted to Tregs



- By day 8, up to 50% of the donor cells in the eye express Foxp3
- Retinoic acid, TGF-β and local Ag recognition in the retina are required for the conversion process
- Tregs converted within the eye are functionally suppressive
  - Inhibit proliferation of fresh IRBP-specific T cells an an Ag-specific assay

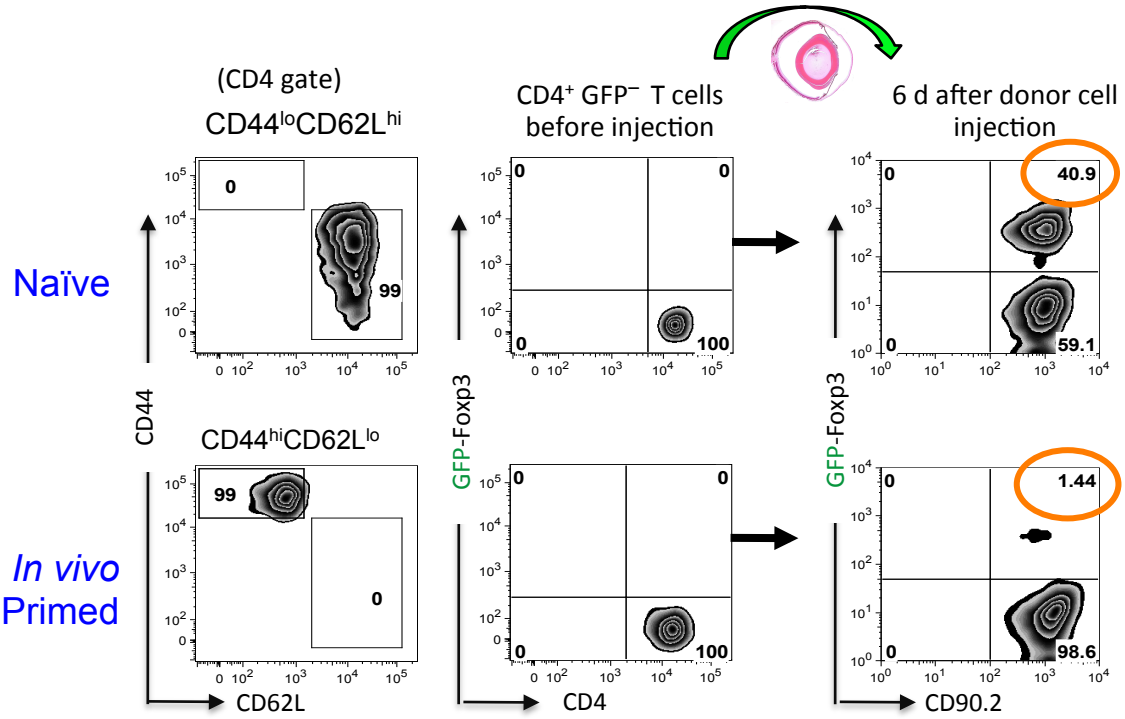
Zhou et al.,JI 2012

Only naïve, but not Ag-experienced T cells can be converted to Tregs within the eye

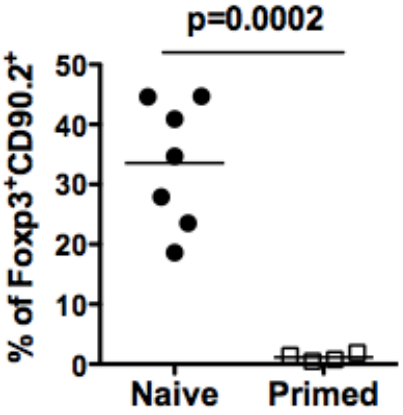
Sorted GFP⁻ T cells from IRBP TCR Tg, FoxP3-GFP reporter, CD90.2⁺ donor mice

–> Naïve: $CD44^{low}CD62L^{high}$ from unimmunized donors

–> Primed: $CD44^{high}CD62L^{low}$ cells from IRBP-immunized donors

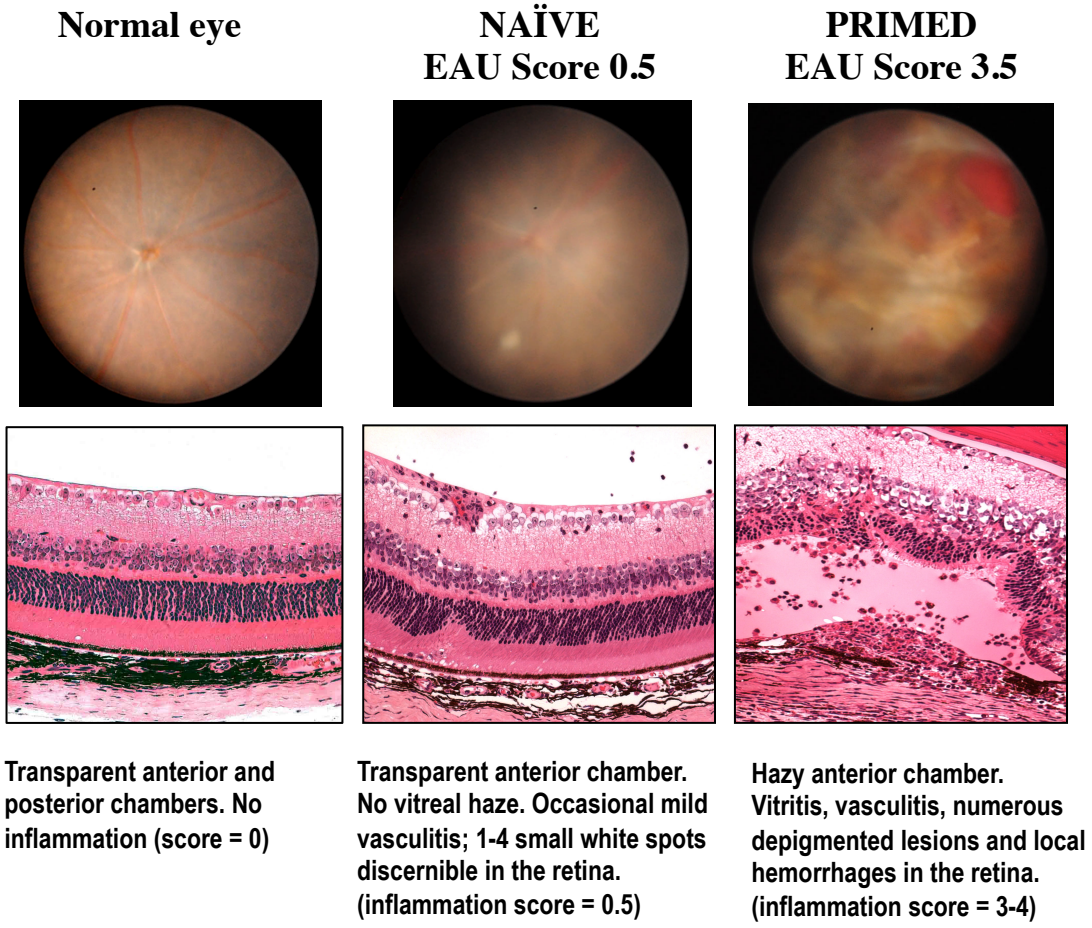


Conversion of T cells to Tregs: 6 individual expts



—> T cells that have acquired effector function outside the eye resist conversion

Unlike naïve T cells, antigen-experienced T cells are not controlled by the inhibitory environment of the eye and induce uveitis



Immune privilege has limitations:

Immune privilege restrains naïve retina-specific cells that may have accidentally made their way into the eye due to a microtrauma or a vascular abnormality.

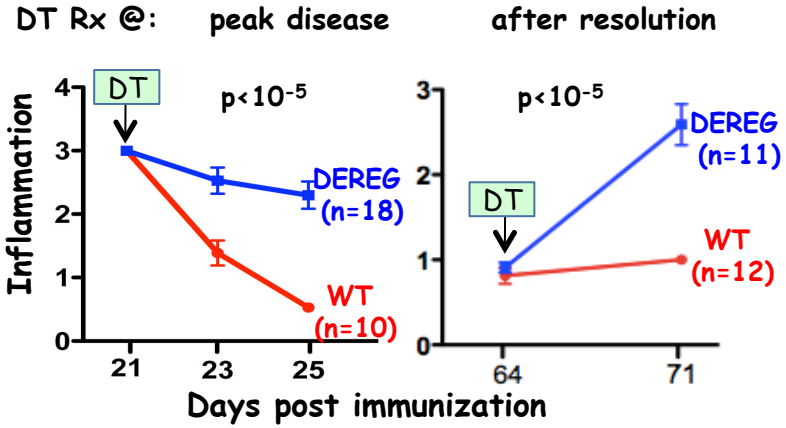
Immune privilege does not control activated retina-specific effector T cells that have been activated outside the eye and are able to actively cross the BRB.

This can help explain why uveitis occurs despite immune privilege

How is uveitis controlled?

- **Tregs coming in from the periphery**
  (and to a lesser extent induced locally)

Depletion of Tregs in Foxp3-DTR mice by diphtheria toxin injection prevents resolution or reactivates disease



Phyllis Silver

- **Bregs – as inducers of Tregs?** (Egwuagu lab)

- **Other regulatory cells (MDSC, etc.)**

Summary I

- Circulating retina-specific T cells, which are 'ignorant' but not 'tolerant' due to inadequate peripheral tolerance, are activated outside the eye to become Th1 or Th17 effectors, either one can fuel disease.

- Activated T cells reach the eye, cross the BRB and recognize their antigen in the tissue. They trigger an inflammatory cascade with massive recruitment of inflammatory leukocytes from the circulation, that manifests as uveitis.

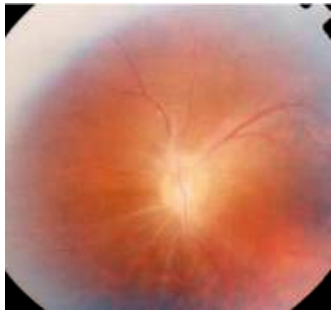
- Although the eye can control naïve T cells and convert them into Tregs, effector T cells resist the ocular inhibitory microenvironment and induce uveitis before local and systemic regulatory mechanisms can step in to control them. This can explain why uveitis occurs despite ocular immune privilege.

- Resolution of active disease and maintenance of remission are brought about by Treg cells and other regulatory cells, which are in part recruited from the periphery and in part may be induced and act locally.

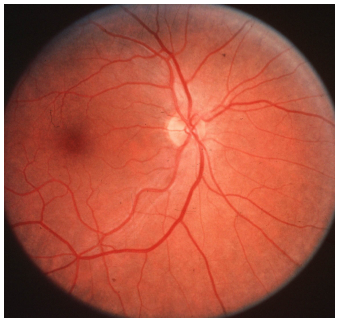
Clinically and immunologically, autoimmune uveitis is a heterogeneous disease

- Varies in clinical appearance and course even when the patients respond to the same retinal antigen(s) [S-Ag, IRBP]
- Both **Th1** and **Th17** cytokines were reported in association with different types of uveitis in patients, potentially helping to explain the heterogeneity
- Due to practical and ethical limitations, causal relationships in humans are hard to prove.

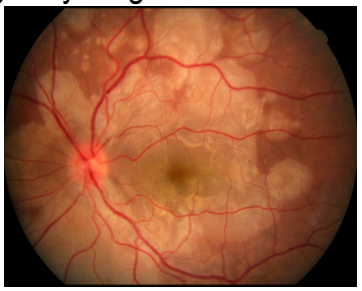
Behcet's disease



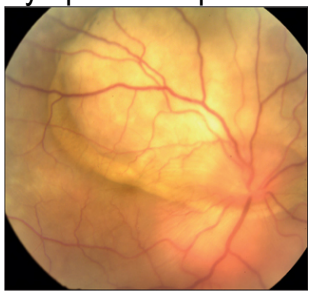
Normal human fundus



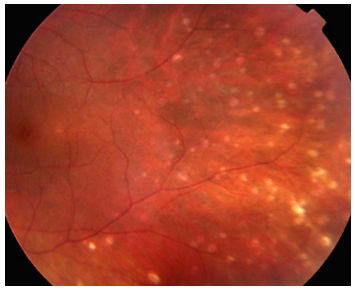
Vogt-Koyanagi-Harada disease



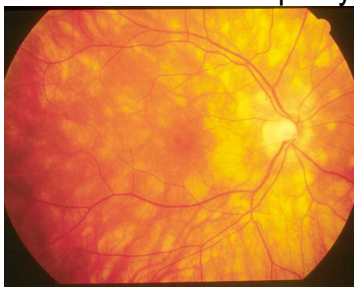
Sympathetic ophthalmia



Ocular Sarcoidosis



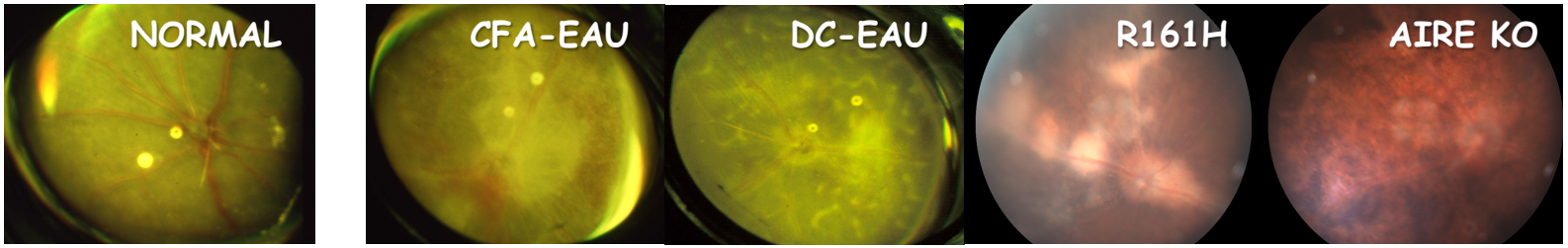
Birdshot chorioretinopathy



No single animal model can reproduce the entire spectrum of human uveitis.

To study this complexity, we and others have developed models of autoimmune uveitis in mice, which differ in clinical appearance and immunological mechanisms, and may represent different aspects of human disease

Models in use in our lab:



Major models of EAU

A. Induced models –> WT mice challenged for disease
bacterial adjuvants required to enhance response

"Classical" EAU: immunization with retinal Ag/CFA

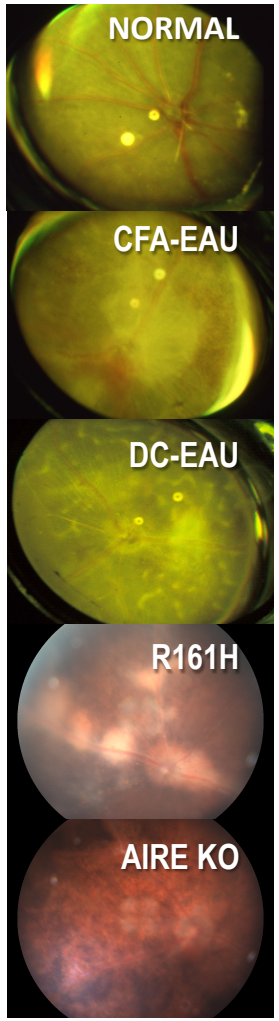
DC-EAU: induced with Ag-pulsed, matured DC

B. Spontaneous models –> genetically altered mice
high frequency of retina-specific T cells

Spontaneous uveitis in **IRBP TCR Tg mice**

Spontaneous uveitis in **AIRE KO mice**

C. Adoptive transfer of activated effector T cells

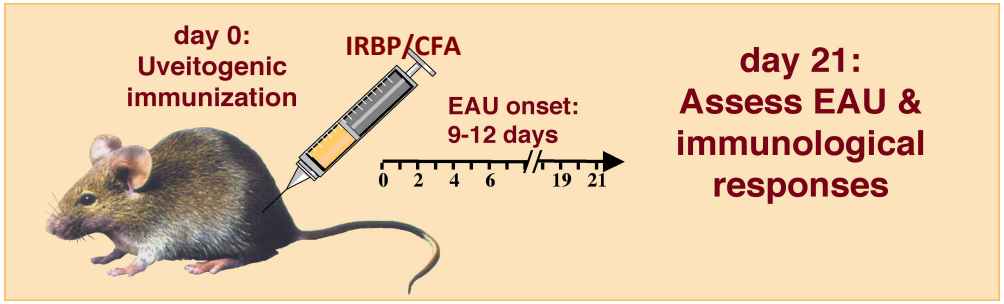


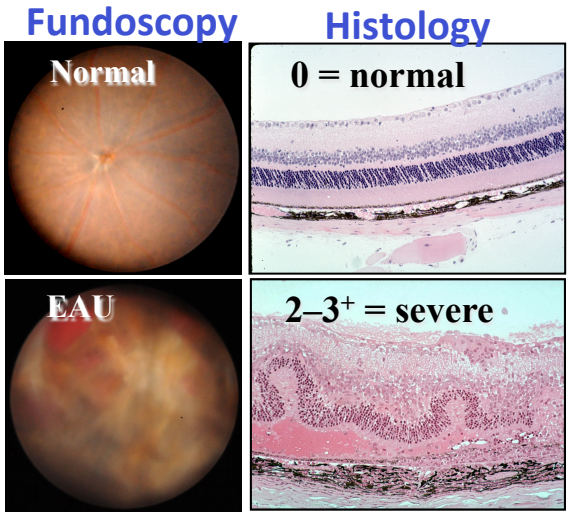
Much of what we know about basic mechanisms of uveitis is based on the "classical" model

1

The "classical" EAU model:
immunization with retinal antigen in CFA

Caspi et al, J. Immunol 1988





Over the last 28 years has been instrumental in unraveling the basic mechanisms of uveitis

The EAU model as a platform for testing new therapeutic approaches

The ability of an immunomodulatory therapy to affect development and/or expression of the experimental disease has often predicted efficacy in a clinical setting

- Cyclosporine A (calcineurin inhibitor)
- Rapamycin (mTOR pathway inhibitor)
- Daclizumab (anti IL-2R = CD25)
- Anakinra (IL-1R antagonist)
- Gevokizumab (anti-IL-1)
- Infliximab, adalimumab (anti-TNF-α)
- Type I interferons (IFN-α, IFN-β)
- Abatacept (CTLA4-Ig: blocks costimulation)
- Rituximab (anti CD20: B cell depletion)
- *Sekukinumab**???** (anti-IL-17)*

"I have come to the conclusion, that a drug is any substance which, when injected into a rodent, produces a paper."


Mary Mattapallil

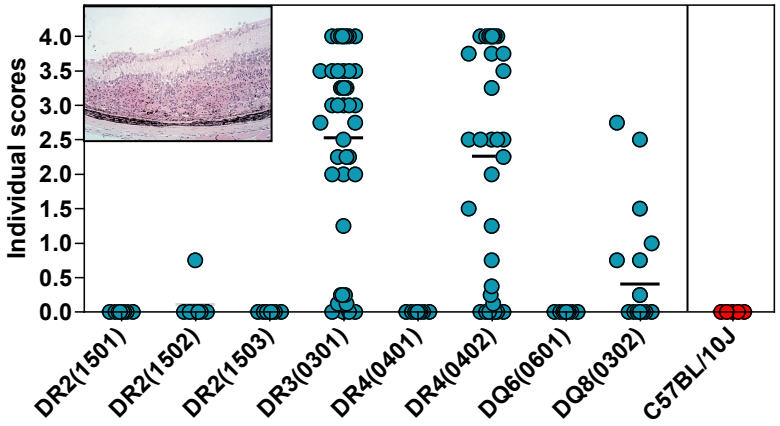

Pina Pennesi

"Humanized" model of EAU
-a variant of the induced model

Pennesi et al, JCI 2003, Mattapallil et al, JI 2011

- HLA class II transgenic mice that lack mouse class II, (Chella David, Mayo Clinic) immunized with S-Ag (arrestin).
- Patients respond to S-Ag, to which most mouse strains are resistant.

DR3, DR4 and DQ8 Tg mice develop severe EAU with S-Ag



HLA Tg mice respond to peptides from the S-Ag sequence that are recognized by lymphocytes of uveitis patients, e.g., Peptide M

Proof of concept

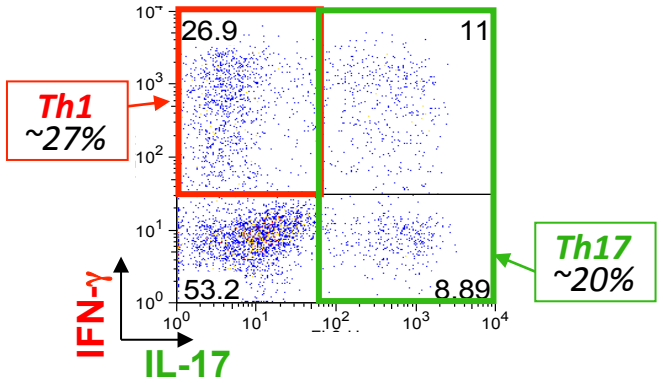
- *A retinal Ag to which patients respond, causes uveitis when presented to the immune system by human MHC molecules*
- Supports an etiological role for retinal antigens in the human disease
- Validates use of the EAU model for study of human uveitis

Cytokine dependence of uveitis

"Classical" EAU: requirement for IL-17

Dror Luger

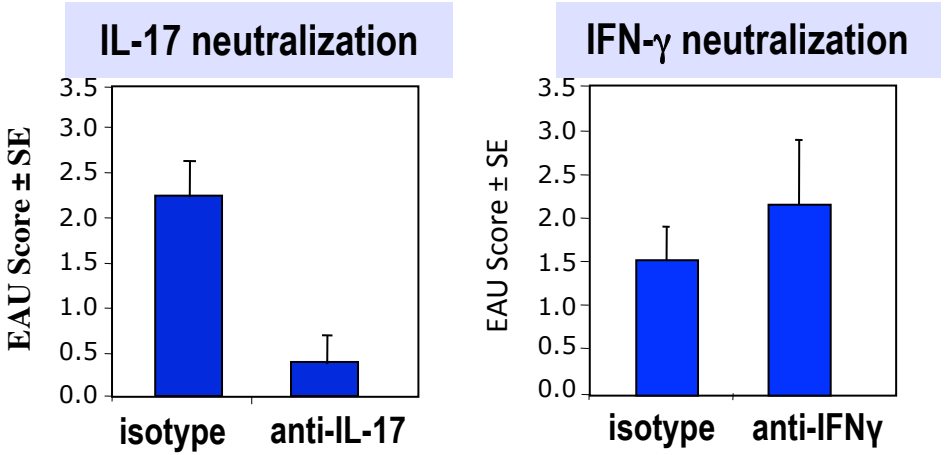
T cells producing IFN-γ (Th1) and T cells producing IL-17 (Th17) infiltrate uveitic eyes in similar quantities



Is Th17 or Th1 more important for pathology?

Neutralization of IL-17, but not of IFN-γ, prevents as well as reverses EAU

The pattern is reproduced in KO mice that lack IFN-γ or IL-17



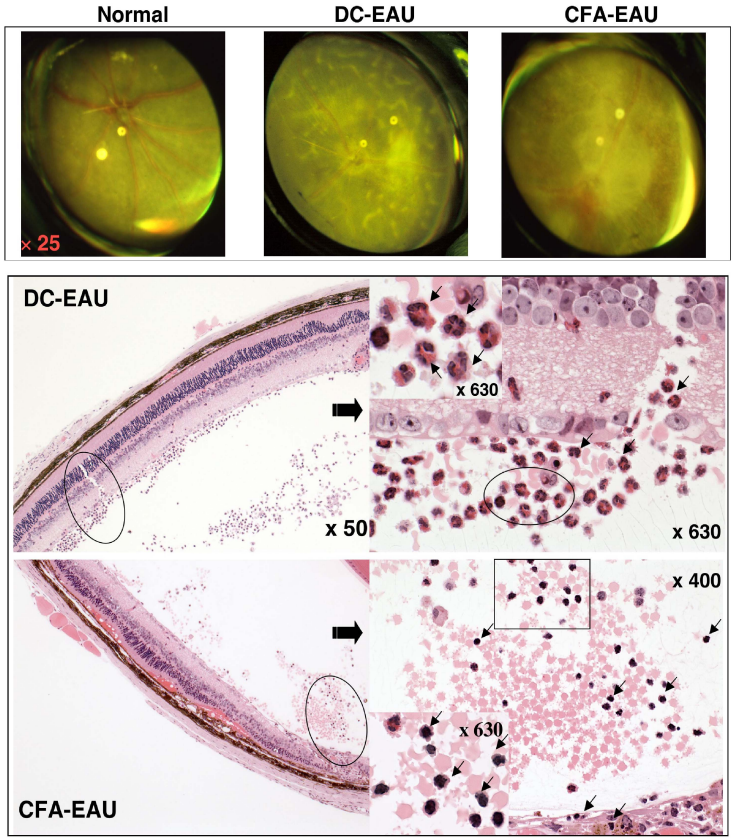
Although both Th1 and Th17 cells are present, the Th17 response appears to be driving pathology

2

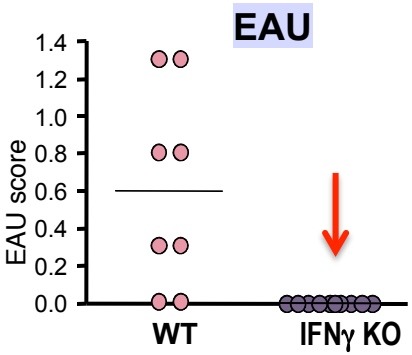
EAU induced by injection of IRBP peptide-pulsed DC: requirement for IFN-γ

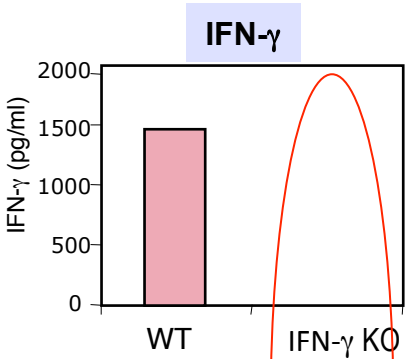
Jun Tang

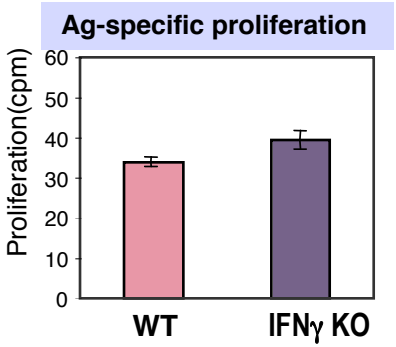
Tang et al, J. Immunol. 2007

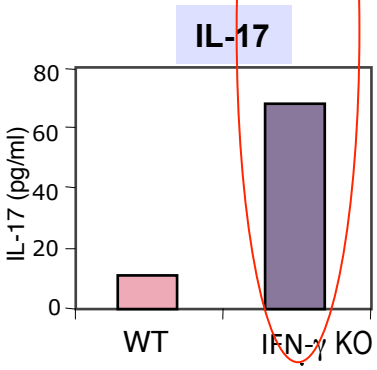


Conditioned DC from WT donors fail to induce EAU in IFN-γ KO recipients









An IL-17 response in the absence of IFN-γ is insufficient to support EAU in this model

➢ **IFN-γ-producing effector T cells (Th1) appear to be required**

3

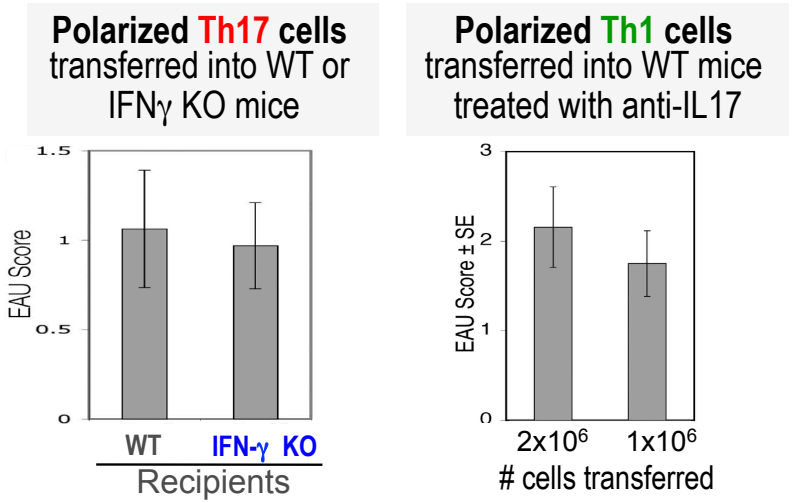
Adoptively transferred EAU model

- **Induced in naïve recipients with activated IRBP-specific effector T cells** that had been isolated from mice immunized for uveitis
- **a model of the effector phase (efferent phase) of disease, after the pathogenic T cells had been primed**
- does not require the use of immunological adjuvants or genetic modification of the recipient

  ⇒ more clinically relevant???

T cells polarized to Th1 or Th17 by activation in presence of a cytokine cocktail of the appropriate composition, are equally pathogenic

⇒ Th1 and Th17 are each a standalone effector capable of driving disease



Luger et al., J. Exp. Med. 2008

The uveitis paradox:

Retina-specific T cells must be **activated** to be able to cross the blood-retinal barrier (BRB) and induce uveitis

BUT...

The retinal antigens are sequestered within the eye behind the BRB as part of ocular immune privilege, and are not available in the periphery to activate T cells.

Only a very small minority of uveitis cases can be explained by trauma to the eye that makes retinal antigens available to the immune system

Where and how do uveitis-inducing lymphocytes first become activated?

Based on anecdotal evidence, microbial involvement in some types of human autoimmune uveitis has been proposed (but never rigorously proven)

- **Klebsiella and HLA-B27 arthritis/uveitis**
  (Ebringer et al, Immunol. Rev. 1985, Kilstra et al., Br. J Opthalmol. 1986)
- **G^- bacteria and reactive arthritis/uveitis (Reiter's Syndrome)**
  (Gaston & Lillicrap, Best Pract Res Clin Rheumatol 2003)
- **Streptococcus Sanguinis and Behçet's disease**
  (Kaneko et al, Eur J Dermatol 2008)
- **Mycobacteria and Sarcoidosis** *(Gupta et al, Eur Respir J 2007)*

To explore these questions experimentally, a spontaneous model of EAU is needed

3

Generation of transgenic mice that express a uveitogenic T cell receptor

Rajeev Agarwal

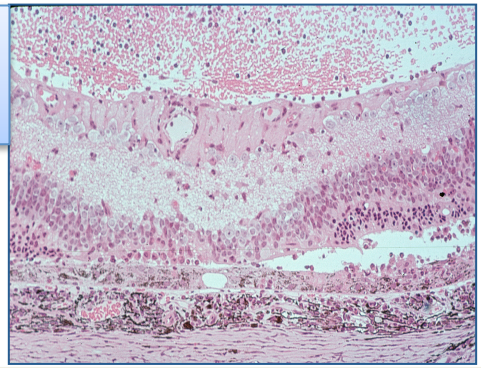
Reiko Horai

WT B10.RIII
(EAU susceptible)

IRBP
in CFA

Expand draining lymph node cells with $IRBP_{161-180}$

Uveitogenic T cell line

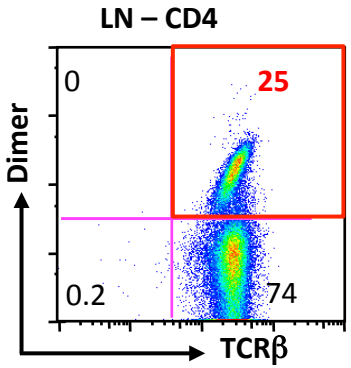


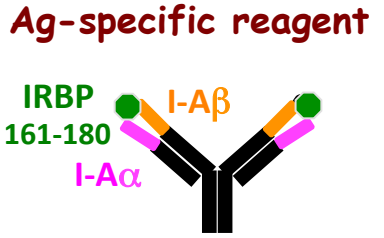
Severe EAU induced by $1x10^6$ T cells from uveitogenic line

- Clone TCR (Vα17/Vβ1)
- Insert TCR α and β chain constructs into B10.RIII oocytes
- Derive IRBP TCR Tg mouse line (R161H)

Horai et al, JAUT 2013

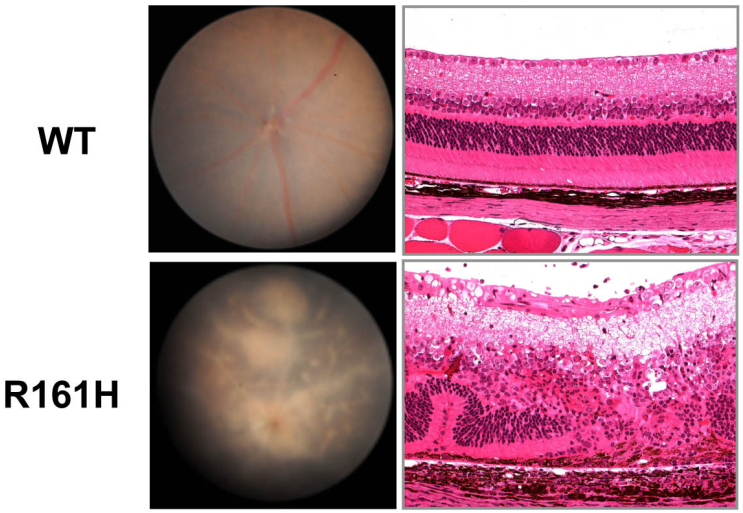
R161H mice: a model of spontaneous uveitis





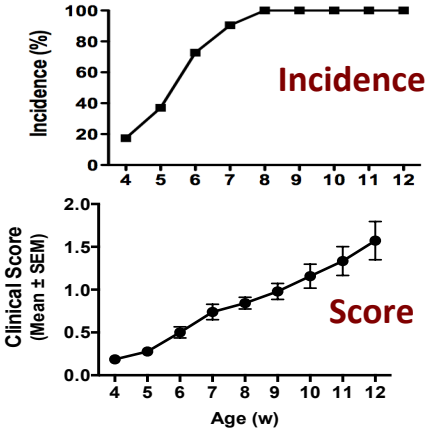
IRBP161-180/I-A^r/IgG1dimer
(Karabekian et al. IOVS 2005)

- IRBP-specific TCR expressed on 20-30% of peripheral T cells
- Spontaneously develop ocular inflammation by 2 months of age
- Both IFN-γ and IL-17 produced by eye-infiltrating T cells

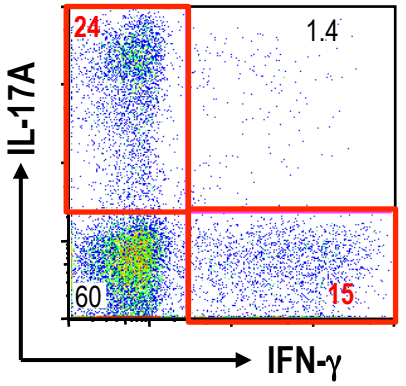


Horai et al, JAUT 2013

Fundus evaluation

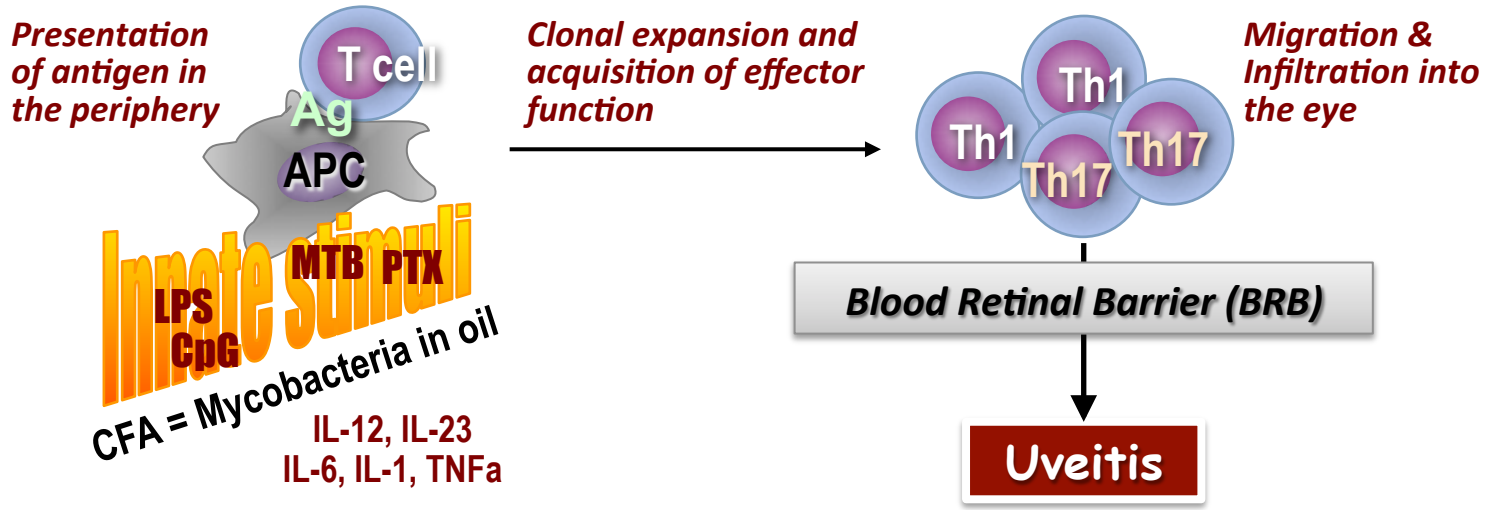


Eye infiltrating cells



Activation and differentiation of uveitogenic T cells requires innate microbial signals

- in the induced EAU model this is provided by CFA



- How are retina-specific T cells activated in spontaneous uveitis?
- Do commensal bacteria have a role in activating these T cells?

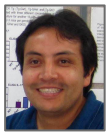
The spontaneous uveitis model permits study of natural triggers of uveitis:

endogenous gut flora as a potential trigger

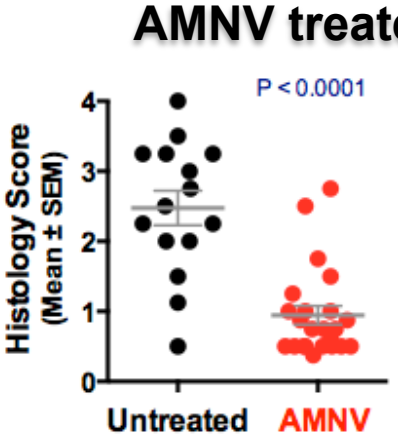
Elimination of commensal microbiota by antibiotic treatment or by rearing in germ-free conditions prevents development of disease

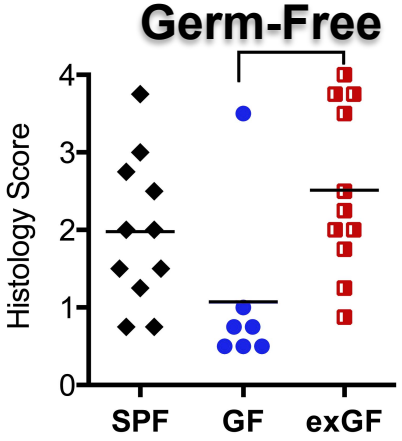
9-12 week old mice:

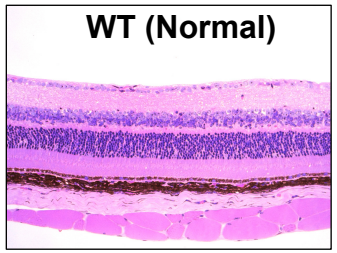
Reiko Horai

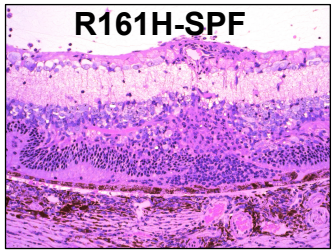


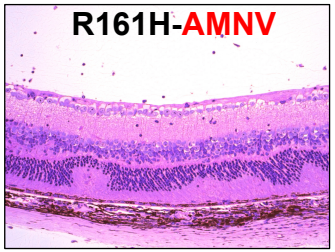
Carlos Zárate Bladés

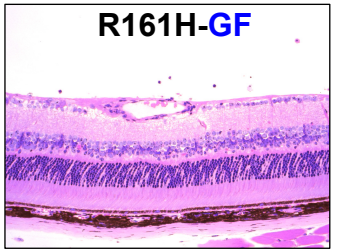








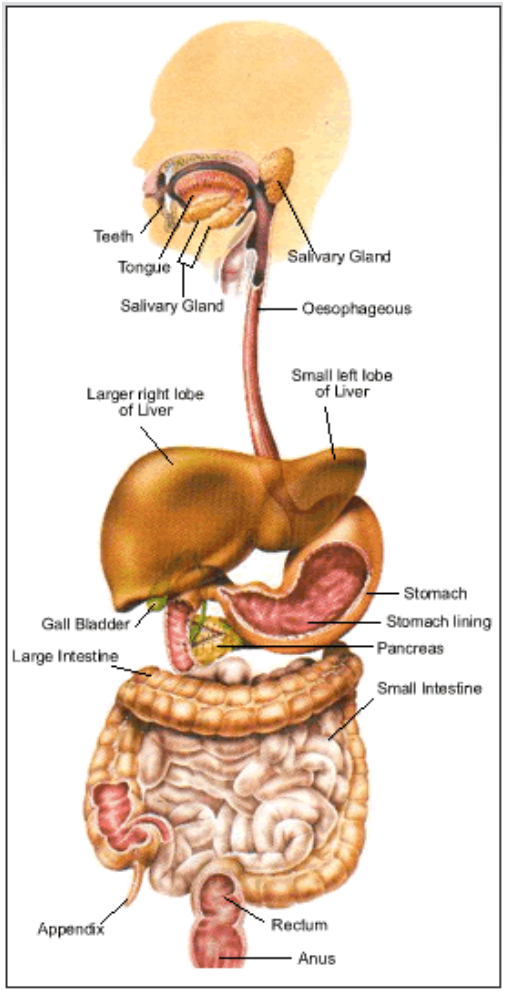


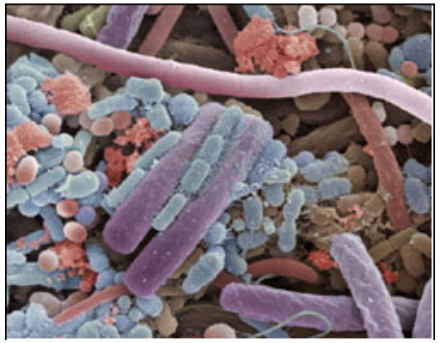


—> Commensal flora contributes to disease

Mechanism???

Colonization by Commensals





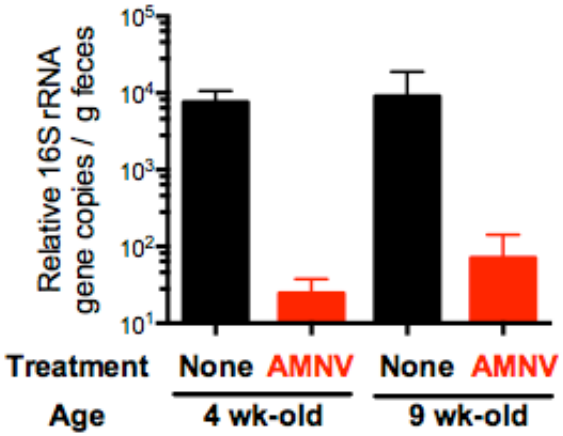
- At least 100 trillion bacteria in the gut; (10x more than all cells in the body) thousands of species
- Inner surface of the gut is similar in area to a tennis court
- Major reservoir of lymphocytes (up to 20% of total depending on species)

Approach: Eliminate endogenous bacterial flora from R161H mice and examine effects on spontaneous uveitis

1) "Poor man's germ-free mice"
(Rakoff-Nahoum et al. Cell, 2004)

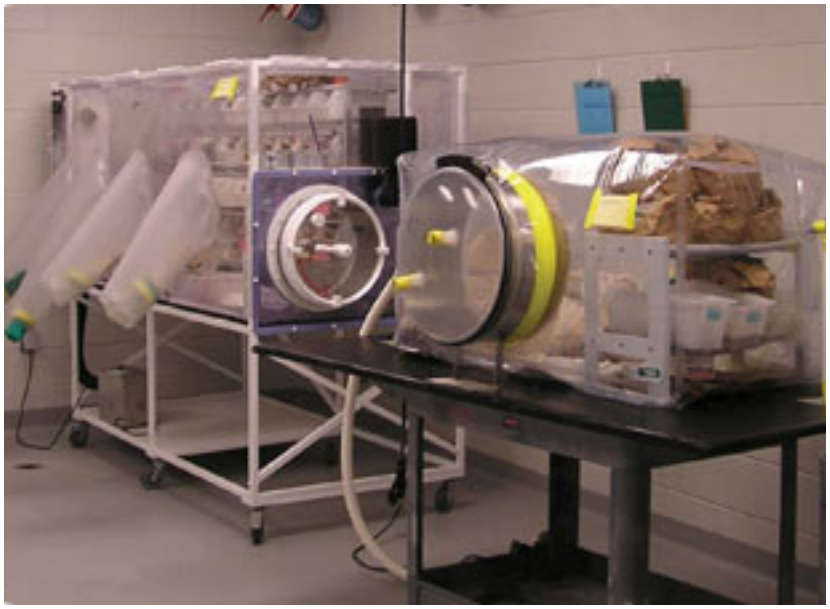
- **A**mpicillin (1 g/L)
- **M**etronidazole (1 g/L)
- **N**eomycin (1 g/L)
- **V**ancomycin (500 mg/L)

Administered in drinking water



Total bacterial mass and flora complexity drastically reduced

2) "Real" germ free mice
(Collaboration w/ Kenya Honda, Japan)



All microbial flora eliminated

SFB

Reiko Horai

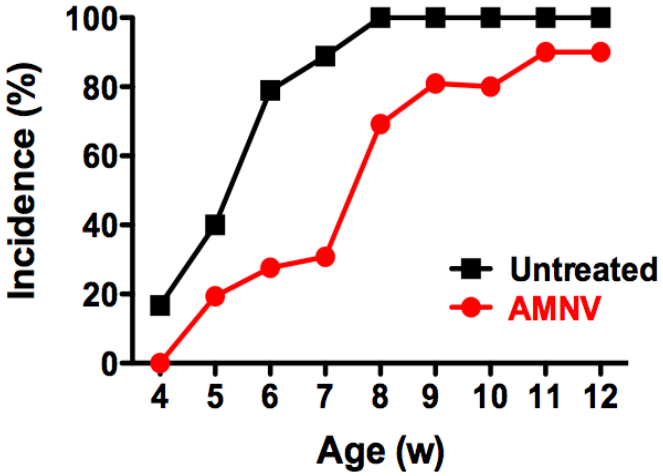
Carlos Bladés

Elimination of commensals delays onset and reduces severity of spontaneous uveitis in R161H mice

"Poor Man's" Germ-Free mice: AMNV

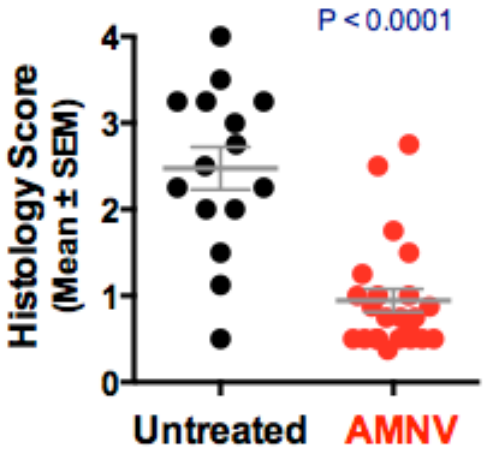
Real Germ-Free mice

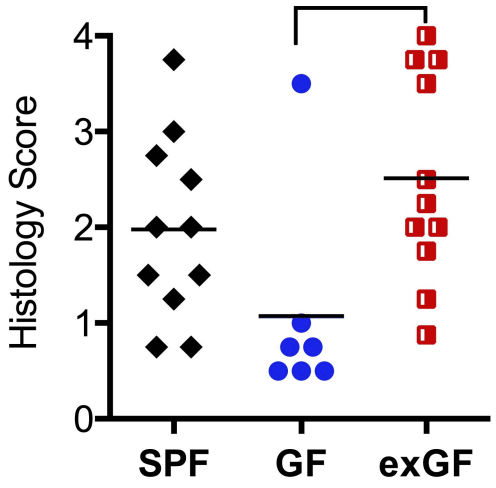
Incidence



Histopathology

(9-12 w)





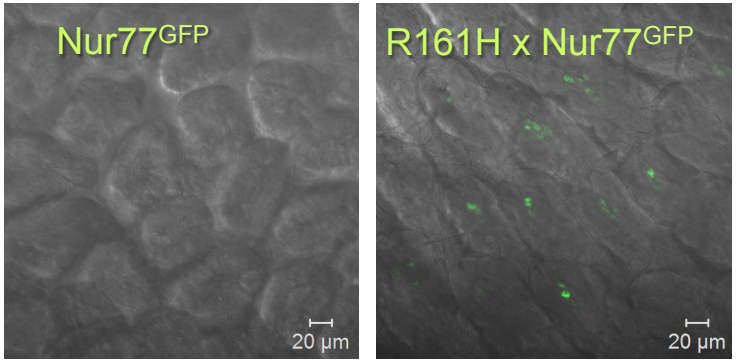
—> Commensal flora contributes to disease

Mechanism???

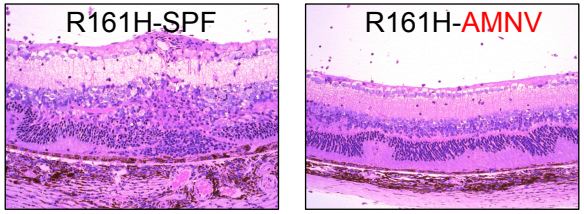
Retina-specific T cells are activated in the intestinal lamina propria by microbial products and acquire effector function

- **T cell activation occurs in the gut of R161H mice and precedes clinical disease**

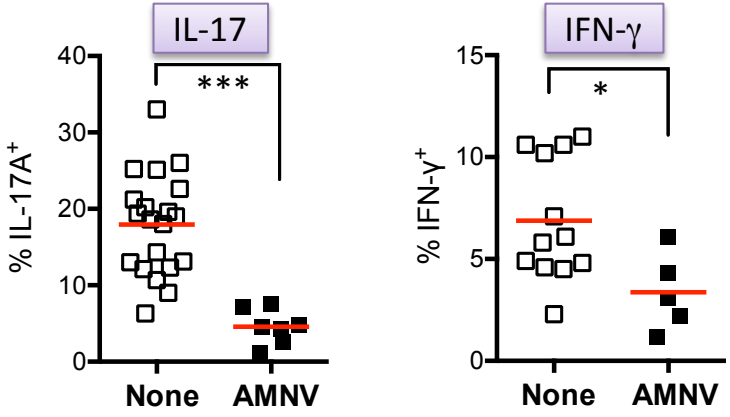
Freshly explanted ileum from Nur77-GFP reporter mice, 17d old



- **Activation is dependent on microbiota (AMNV) and results in induction of Th1 and Th17 cells that are associated with disease**

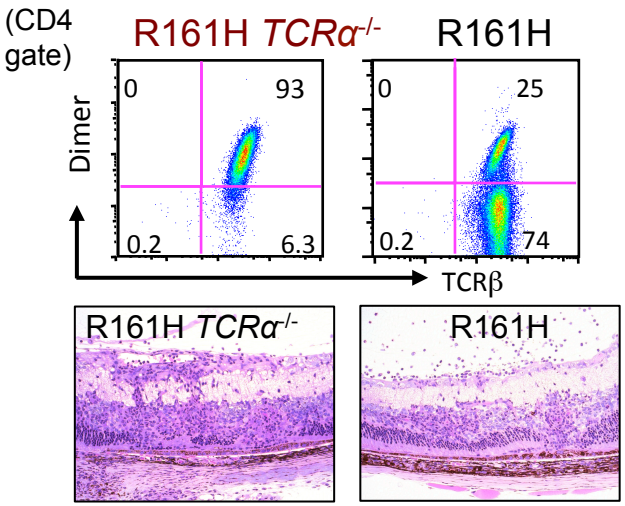


Lamina propria T cells (CD4 gate)

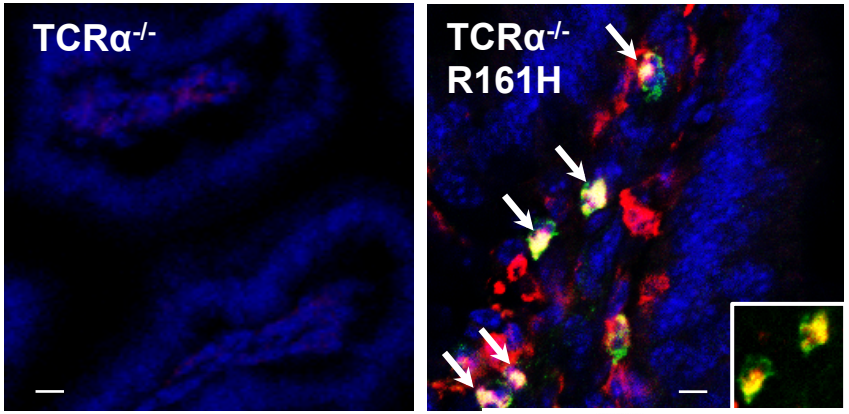


Is IL-17 or IFN-γ more important for pathology?

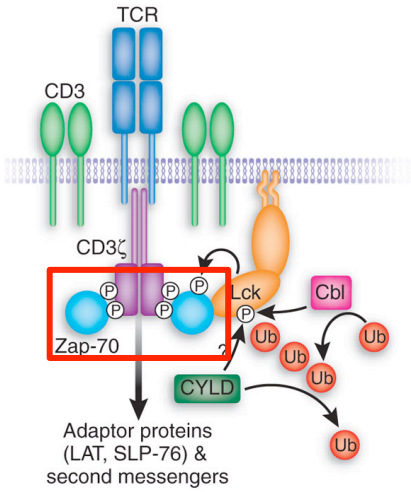
Activation of retina-specific T cells in the intestine involves an antigen mimic that signals through the retina-specific TCR



Tyrosine phosphorylation of Zap70 (Tyr319) indicates engagement of the retina-specific R161 TCR



—> there may also be a role for bacterial adjuvant effects

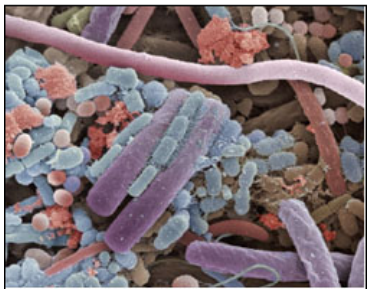


—> Implications for etiology of human uveitis

—> Implications for etiology of autoimmune diseases in general

Commensal microorganisms provide a trigger and/or an amplification signal to autoreactive effector T cells that drive spontaneous uveitis.

Signaling through the R161H TCR by a mimic antigen from gut microbes appears to be involved.



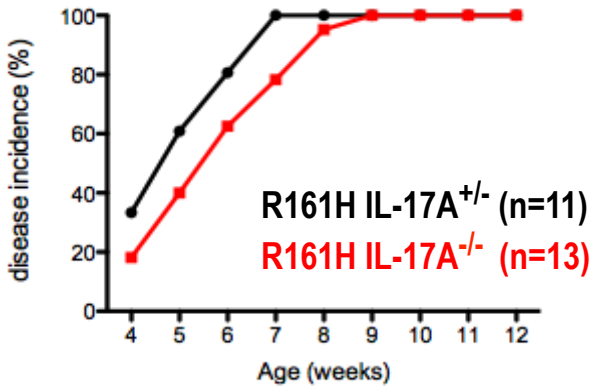
- Implications for pathogenesis of human uveitis?
- How general is T cell activation through an autoreactive TCR by gut flora??? —> can it be involved also in other autoimmune diseases?

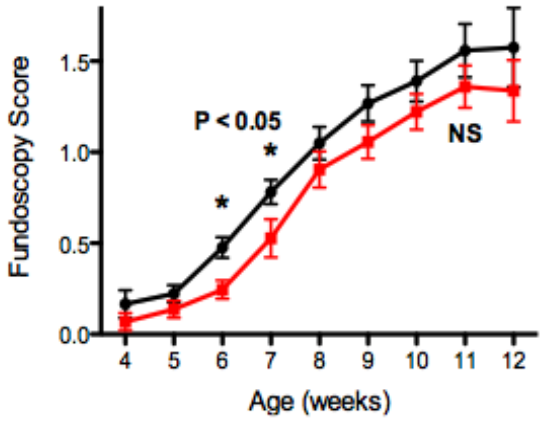
Jun Chen

IFN-γ, and not IL-17, is necessary for full development of uveitis in R161H mice

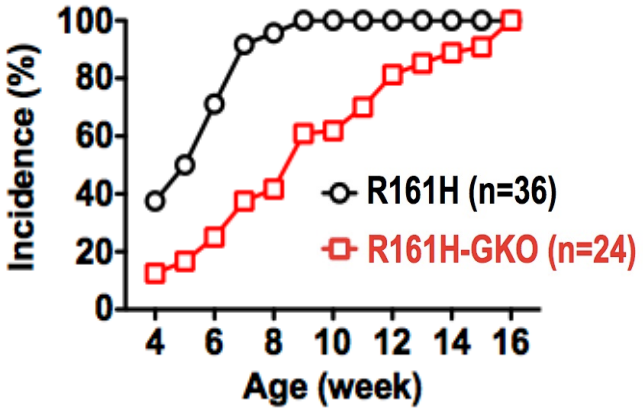
Clinical disease progression by fundus examination

IL-17 deficiency – minimal effect

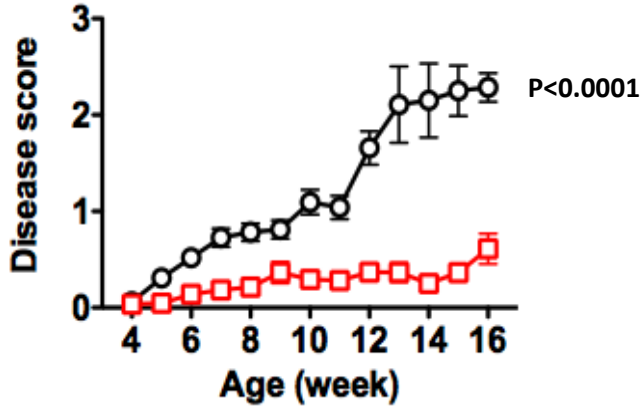




IFN-γ deficiency – major effect



Incidence



Severity

Summary II

Mouse models of uveitis differ in their effector dependence

A. Induced models	Dominant T cell lineage
”Classical" EAU: immunized with IRBP/CFA *Caspi et al, J. Immunol 1988; Luger et al., J. Exp. Med. 2008*	Th17
DC-EAU: induced with IRBP-pulsed mature DC *Tang et al, J. Immunol. 2007*	Th1
B. Spontaneous models	
Spontaneous EAU in IRBP TCR Tg mice *Horai et al., J. Autoimmun 2014*	Th1
Spontaneous uveitis in AIRE KO mice *Anderson et al., Science 2002*	not studied*
C. Adoptive transfer of cultured T cells	Th1 or Th17

*AIRE KO mice have other autoimmune pathologies that appear Th1 dependent

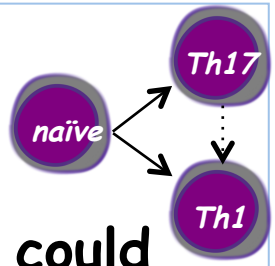
Conclusions

Uveitis can be Th17-driven, or Th1-driven

- **Either of these responses can be dominant in pathogenesis, depending on the model.**
- **Both Th1 and Th17 may be pertinent to human uveitis and may help explain disease heterogeneity**
- **Effector dominance is affected by inductive events** at the time of initial Ag exposure: innate stimuli create a polarizing cytokine milieu for priming of the Ag-specific T cell.
  - Strong bacterial stimuli (CFA) result in a Th17-dominant disease
  - When disease is induced without CFA (Ag-pulsed mature DC, spontaneous uveitis), Th1 is dominant.
- **In human disease the inductive events are usually unknown.**

Effector dominance has implications for therapy:
If animal models are relvant, targeting Th1 may be effective against some types of uveitis, while others may benefit from anti-Th17 therapy.

Potential problem: **Although one effector response may predominate, both are always present.**
In view of the inherent plasticity of effector T cell responses, suppressing one effector pathway could over time shift the response towards the other, without reducing pathology (possibly explaining variable results of anti-IL-17 therapy in uveitis?)



Take-home message: **Will targeting a single effector pathway in uveitis be sufficient? - Consider therapeutic approaches that target *both* Th1 and Th17 to offset effector T cell plasticity**. (e.g., IL-12/23p40, IL-27p28)

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